Both VI and VII are ineffective as housefly chemosterilants though in this species VI is reported to be metabolised to PMPA.[5] Assuming that the transformation of VI to PMPA proceeds reductively *via* the methylol (II) in both rats and houseflies, the biological activity of HMPA in these species must be associated with the molecule itself and not be due to the methylol (II).[3]

METHODS

HMPA and PMPA were administered separately in the drinking water (0·05%) to groups of six female Wistar rats for 5 weeks, the filtered urine collected and frozen in solid carbon dioxide. The urine (approximately 1 l. in each case) was extracted and chromatographed on Whatmans SG-31 Chromedia as previously described[3] to remove HMPA, PMPA and *N, N, N′, N″*-tetramethylphosphoramide. The columns were then eluted with methanol and the extracts chromatographed on freshly activated[3] preparative TLC plates (1·5 mm) of silica gel G in solvent A (chloroform–ethanol 3–1). From HMPA-administered urine, the area R_f 0·44–0·55 (developed yellow with molybdate reagent[6]) was eluted with methanol, evaporated *in vacuo*, dissolved in chloroform and purified on a small cellite column. This gave a pale yellow oil (23 mg) characterised as *N*-formyl-pentamethylphosphoramide (VI) by reference to an authentic sample.[5] M$^+$ 193·098782; $C_6H_{16}N_3PO_2$ requires M$^+$ 193·098014 (error 3 p.p.m.). IR (chloroform) 1695 cm^{-1} (CHO). R_f 0·52 on 250 μ silica gel G in solvent A.

From PMPA-administered urine the area R_f 0·30–0·40 was similarly extracted from preparative TLC plates as a yellow gum (18 mg) and characterised as *N′*-formyl-*N, N, N′, N″*-tetramethylphosphoramide (VII) by reference to an authentic sample.[5] M$^+$ 179·081778; $C_5H_{14}N_3PO_2$ requires M$^+$ 179·082364 (error 3 p.p.m.). IR (chloroform) 1690 cm^{-1} (CHO). R_f 0·41 on 250 μ silica gel G in solvent A.

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REFERENCES

1. H. JACKSON and A. W. CRAIG, *Nature, Lond.* **212**, 86 (1966).
2. P. H. TERRY and A. B. BORKOVEC, *J. Med. Chem.* **10**, 118 (1967).
3. A. R. JONES and H. JACKSON, *Biochem. Pharmac.* **17**, 2247 (1968).
4. S. C. CHANG, P. H. TERRY, C. W. WOODS and A. B. BORKOVEC, *J. Econ. Ent.* **60**, 1623 (1967).
5. P. H. TERRY and A. B. BORKOVEC, *J. Med. Chem.* **11**, 958 (1968).
6. C. S. HANES and F. A. ISHERWOOD, *Nature, Lond.* **164**, 1107 (1949).

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Alteration in tyramine metabolism by ethanol

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PRETREATMENT of animals with ethanol alters the pattern of metabolism of the biogenic amines serotonin and norepinephrine.[1–3] These amines are deaminated by monoamine oxidase with the formation of an aldehyde intermediate which is either oxidized by aldehyde dehydrogenase to the corresponding acid or reduced by alcohol dehydrogenase to the corresponding alcohol. Ethanol causes an increase in the production of the alcohol and a decrease in the production of the acid from an administered dose of serotonin or norepinephrine. In the course of an investigation of the metabolism of tyramine-1-^{14}C in the rat we have studied the effect of ethanol on the metabolism of this

amine and have found differences after ethanol treatment similar to those found with serotonin and norepinephrine.

Male Sprague–Dawley rats weighing 200 g were maintained in metabolism cages (Acme Metal Products). They were given water *ad lib.* but no food during the first 24 hr of the experiment. The experimental group received ethanol (2 g/kg as a 25% solution in saline) intraperitoneally and 20 min later tyramine-1-^{14}C HBr (1·9 mg/kg, 5·3 mc/m-mole in 0·5 ml saline) also intraperitoneally. The control group received the same dose of tyramine-1-^{14}C HBr but no ethanol. Urine for the identification and determination of metabolites was collected at 3, 6, 9, 12 and 24 hr after dosage, brought to pH 1 with 2 N HCl and stored at −10°. Under these conditions tyramine added to urine is unaltered. Collection of urine was continued up to 96 hr by which time 97 per cent of the administered radioactivity had been excreted.

The metabolites were identified by color reactions and R_f values on paper (Whatman 3 MM) and thin-layer (Silica Gel H, Brinkman Instruments, Inc.) chromatography in five solvent systems (see Table 1). Conjugates were further identified by the R_f values on paper and thin-layer chromatography

TABLE 1. R_f VALUES AND COLOUR REACTIONS OF SOME METABOLITES OF TYRAMINE-^{14}C

	R_f values Paper chromatography					Thin-layer chromatography
Metabolite	A*	B	C	D	E	E
p-Hydroxyphenylacetic acid	0·34	0·84	0·40	0·71	0·55	0·60
p-Hydroxyphenylacetyl glycine	0·27	0·78	0·04	0·57	0·28	0·10
Tyrosol	0·84	0·83	0·40	0·76	0·65	0·50
N-Acetyltyramine	0·84	0·84	0·42	0·82	0·68	0·30

* Solvent systems: A—1-butanol:2-propanol:ammonia:water (3:1:1:1); B—1-butanol:acetic acid, glacial:water (4:1:1); C—benzene:acetic acid, glacial:water (2:2:1, upper phase); D—chloroform: acetic acid, glacial:water (2:2:1, lower phase); E—benzene: propionic acid:water (2:2:1, upper phase).

after hydrolysis with β-glucuronidase, aryl sulfatase or HCl. The identification of *p*-hydroxyphenylacetyl glycine-^{14}C and the sulfate and glucuronide conjugates of tyrosol-^{14}C and *N*-acetyltyramine-^{14}C was confirmed by isotope dilution of the hydrolysates and recrystallization to constant specific activity after specific enzymatic or acid hydrolysis.

Metabolites in the urine collected during the first 24 hr were separated by two-dimensional chromatography of aliquots (0·1–0·3 ml) of urine on Whatman 3 MM paper in solvents A and B (see Table 1). The radioactive areas detected by autoradiography were cut out, eluted and the radioactivity measured by liquid scintillation (Nuclear Chicago Model 720 liquid scintillation counter). The amounts of *p*-hydroxyphenylacetic acid-1-^{14}C, tyrosol-1-^{14}C (*p*-hydroxyphenylethanol) and *N*-acetyltyramine-1-^{14}C were confirmed by isotope dilution and recrystallization to constant specific activity.

The urinary excretion of radioactivity following a dose of tyramine-1-^{14}C is shown in Fig. 1. Ninety-three per cent of the radioactivity is excreted in the first 9 hr and excretion is virtually complete at 24 hr. Ethanol pretreatment causes no significant difference in either the rate of excretion of radioactivity or in the total amount excreted in the urine. The effect of ethanol on the formation of *p*-hydroxyphenylacetic acid, *p*-tyrosol and their conjugates from tyramine is shown in Fig. 2. Ethanol causes a 16 per cent decrease in the amount of free and conjugated *p*-hydroxyphenyacetic acid-1-^{14}C excreted, the effect being most marked (a decrease of 42 per cent, $P < 0{\cdot}001$) in the excretion of *p*-hydroxyphenylacetyl glycine-^{14}C. There is a 20-fold increase in the excretion of tyrosol-^{14}C, the most profound effect being seen in tyrosol sulfate. Free tyrosol, which comprises less than 0·2 per cent of the injected radioactivity, is unaltered. Free and conjugated *N*-acetyltyramine is also unaltered by ethanol pretreatment. The decrease in free and conjugated *p*-hydroxyphenylacetic acid and the increase in conjugated tyrosol represent, respectively, 14·99 per cent and 15·12 per cent of the injected radioactivity.

The first step in the principal metabolic pathway of tyramine is its oxidative deamination to *p*-hydroxyphenylacetaldehyde. Ethanol causes a large increase in the reduction of this aldehyde to

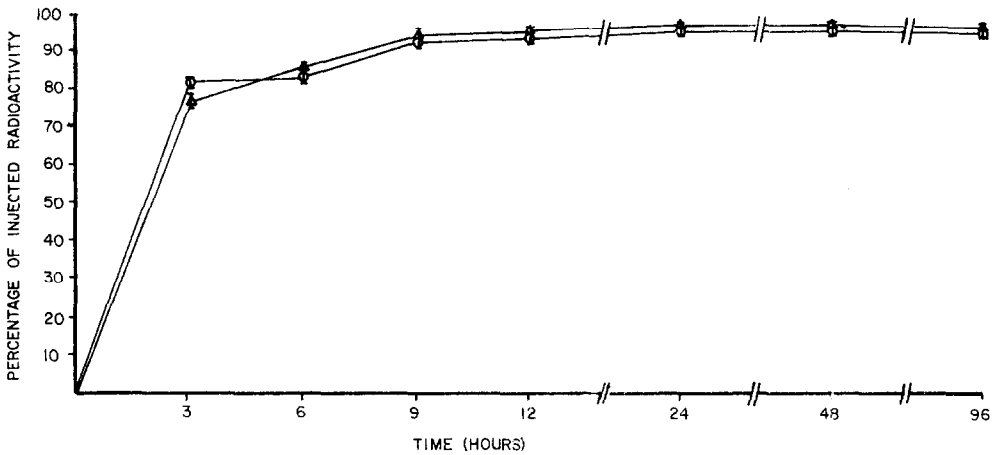

FIG. 1. Urinary excretion of radioactivity by rats given tyramine-1-^{14}C HBr (1·9 mg/kg) intraperitoneally. ○—○, without ethanol pretreatment; △—△, after ethanol 2 g/kg. Results are the means ± S.E.M. for groups of four animals.

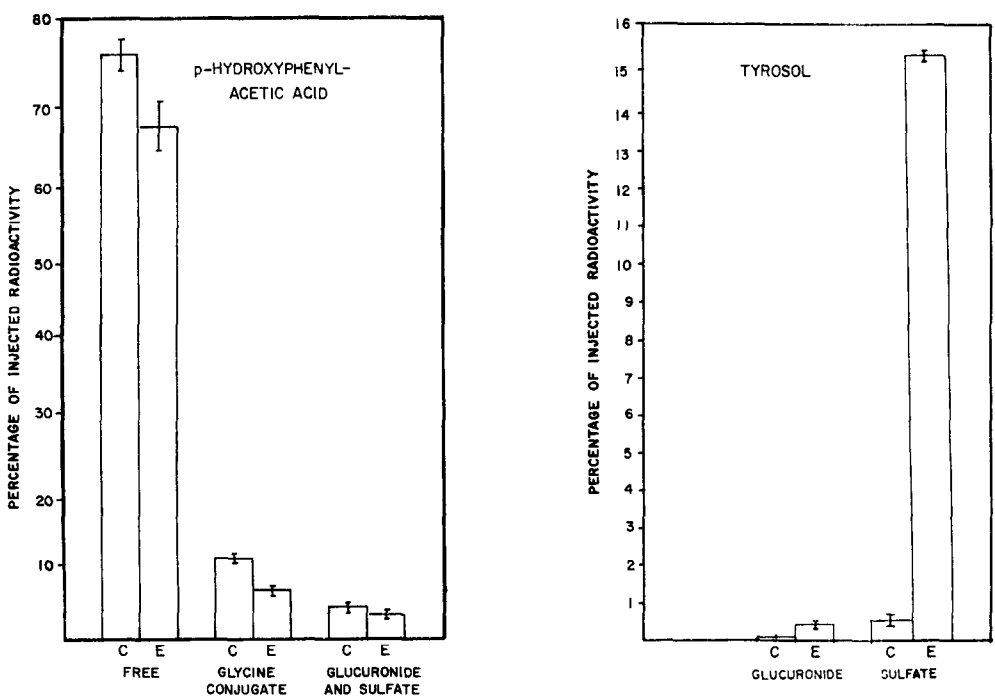

FIG. 2. Metabolites of tyramine-1-^{14}C HBr (1·9 mg/kg intraperitoneally) with (E) and without (C) ethanol pretreatment (2 g/kg intraperitoneally). Results are means ± S.E.M. for groups of four animals.

tyrosol and a corresponding decrease in its oxidation to *p*-hydroxyphenylacetic acid. These results indicate that tyramine metabolism is altered by ethanol in the same way as the metabolism of serotonin and norepinephrine.[1-3] An increase in the NADH/NAD ratio caused by ethanol which increases the reduction of the intermediate aldehyde and decreases its oxidation, and a competitive inhibition of aldehyde dehydrogenase by acetaldehyde have been proposed as possible mechanisms for this effect.[1] The results also suggest a specific effect of ethanol on glycine conjugation, as the decrease in the glycine conjugate of *p*-hydroxyphenylacetic acid is more than twice as great as the decrease in excretion of the free acid. In this connection the observation of Dalgliesh *et al.*,[4] that a large dose of ethanol (6 g/kg B.W.) causes a marked decrease in the excretion of hippuric acid is noteworthy. The increase in tyrosol is represented largely by an increase in the sulfate conjugate there being no increase in free tyrosol and only a 4-fold increase in tyrosol glucuronide. This contrasts with the findings of Davis *et al.*[1] on the effect of ethanol on the metabolism of serotonin-^{14}C in man where a much larger increase in the glucuronide than in the sulfate conjugate of 5-hydroxytryptophol was found.

No change was found in the amount of tyramine-^{14}C excreted as *N*-acetyltyramine. This is in agreement with the observations from this laboratory that ethanol pretreatment does not alter the acetylation of sulphanilamide* in spite of the findings of Ammon *et al.*[5, 6] that acetaldehyde forms a thiohemiacetal with coenzyme A which inhibits its transacetylating ability.

* P. J. Creaven and M. K. Roach, unpublished observations.

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REFERENCES

1. V. E. DAVIS, H. BROWN, J. A. HUFF and J. L. CASHAW, *J. Lab. clin. Med.* **69**, 132 (1967).
2. A. FELDSTEIN, H. HOAGLAND, K. WONG and H. FREEMAN, *Q. Jl Stud. Alcohol* **25**, 218 (1964).
3. V. E. DAVIS, H. BROWN, J. A. HUFF and J. L. CASHAW, *J. Lab. clin. Med.* **69**, 787 (1967).
4. C. E. DALGLIESH, E. C. HORNING, M. G. HORNING, K. L. KNOX and K. YARGER, *Biochem. J.* **101**, 792 (1966).
5. H. P. T. AMMON, C.-J. ESTLER and F. HEIM, *Biochem. Pharmac.* **16**, 789 (1967).
6. H. P. T. AMMON, F. HEIM, C. J. ESTLER, G. FICKEIS and M. WAGNER, *Biochem. Pharmac.* **16**, 1533 (1967).

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Rapid clearance of isosorbide dinitrate from rabbit blood—Determination by gas chromatography*

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IN A RECENT communication Dietz[1] demonstrated, by comparison of R_f values on thin-layer chromatograms, that the metabolites of isosorbide dinitrate (ISDN) in the dog and in man are isosorbide-2-mononitrate and isosorbide-5-mononitrate. DiCarlo *et al.*,[2] studying the biotransformation

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